Characteristics of the spermatozoon of *Cosmopolites sordidus* (Coleoptera: Curculionidae)

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ABSTRACT

The ultrastructure of the spermatozoon of *Cosmopolites sordidus* is similar to that described for other beetles. The acrosome consists of three structures: the perforatorium, the acrosomal vesicle and an extra-acrosomal layer. It is embedded in the nucleus which contains dense, homogeneous chromatin. The flagellum has the typical 9+9+2 microtubule arrangement, two mitochondrial derivatives and two accessory bodies. However, the implantation of these organelles is not in agreement with the description given for the subfamily Rhynchophorinae to which this species belongs. A revision of these characters is needed.

RÉSUMÉ

Caractéristiques du spermatozoïde de *Cosmopolites sordidus* (Coleoptera: Curculionidae)

L'ultrastructure du spermatozoïde de *Cosmopolites sordidus* est similaire à celle décrite pour les autres Coléoptères. L'acrosome est composé de trois structures: le perforatorium, la vésicule acrosomienne et une couche extra-acrosomienne. Il est enchâssé dans le noyau qui contient une chromatine dense et homogène. Le flagelle a la structure typique 9+9+2, deux dérivés mitochondriaux et deux corps accessoires. Toutefois, l'implantation de ces organites ne correspond pas à la description donnée pour la sous-famille des Rhynchophorinae à laquelle cette espèce appartient. Une révision de ces caractères est nécessaire.

Ultrastructural investigations have furnished valuable contributions in relation to the phylogenetic study of many animal groups, including insects. JAMIESON proposed the term spermiocladistics for the use of spermatozoon ultrastructure for phylogenetic reconstruction [8]. Many publications exist along this line; as examples we can cite those dealing with the Chrysomelidae [2] and the superfamily Curculionoidea [5].

While spermatozoal characteristics have been used to define the phylogenetic position of various coleopterans, the position of the *Cosmopolites sordidus* spermatozoon, with regard to its ultrastructural characteristics, is not clear.

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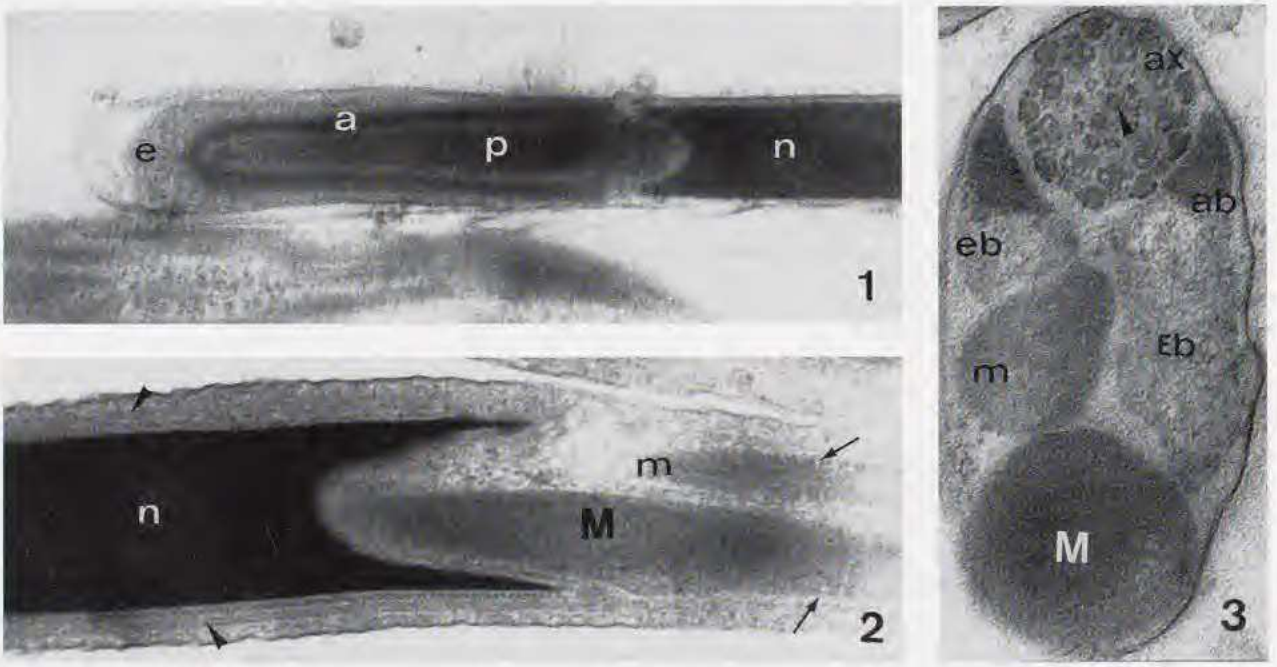
MATERIAL AND METHODS

Adult males were anaesthesized, dissected and their testes fixed by immersion in 3% glutaraldehyde in 0,1 M phosphate buffer at pH 7.2, for 3 hours, post fixed in osmium tetroxide 2% in the same buffer for 1 h, all solutions maintained at 4°C. They were dehydrated with an ethanol series, followed by acetone and embedded in Epon 812. Ultrathin sections were examined with a Zeiss EM 902 electron microscope, after staining with uranyl acetate and lead citrate.

RESULTS

In *Cosmopolites sordidus*, the acrosome measures 1 µm in length and presents three structures: the extra-acrosomal layer, the acrosomal vesicle and the perforatorium. This last structure has its base resting in a concavity of the anterior nuclear surface (Fig. 1).

The nucleus measures 21 µm in length and is totally filled with homogeneous, compact chromatin. The nuclear base forms a cavity which is occupied by the anterior tip of the major mitochondrial derivative (Fig. 2). The flagellum consists of the axoneme, two mitochondrial derivatives and two accessory bodies. In transverse section, the flagellum is oval, with the axoneme and major mitochondrial derivative occupying opposite ends. The axoneme follows the 9+9+2 pattern, with nine accessory microtubules filled with dense material surrounding the nine doublets and two central microtubules, of which, in a perfectly positioned axoneme, the left one is also dense (Fig. 3).



FIGS 1-3 — Spermatozoon of *Cosmopolites sordidus*. **1:** Longitudinal section of the acrosome. The nucleus (n), extra-acrosomal layer (e), acrosomal vesicle (a) and perforatorium (p) can be identified. x 70 000. **2:** Longitudinal section of a late spermatid with the nucleus (N), major mitochondrial derivative (M), minor mitochondrial derivative (m), the expansion of accessory bodies (ab), the major expansion (Eb) and minor expansion (eb) of the accessory bodies. Arrows show the mitochondrial cristae and the arrow heads, the microtubules. x 46 800. **3:** Transverse section of the flagellum, including the axoneme (ax), large mitochondrial derivative (M), minor mitochondrial derivative (m), the major expansion (Eb) and minor expansion (eb) of the accessory bodies (ab). The arrow head indicates the central microtubule not filled with electron dense material. x 82 000.

The major mitochondrial derivative has a diameter equal to twice that of the minor derivative and is almost entirely occupied by material in a paracrystalline arrangement (Fig. 3), although small cristae can still be found (Fig. 2). Cristae are larger in the minor mitochondrial derivative.

The accessory bodies lie parallel to the axoneme, between this structure and the mitochondrial derivatives. Each body has a dense region and a less compact expansion, which is larger for one of the accessory bodies (Fig. 3).

DISCUSSION

The acrosome of *Cosmopolites sordidus* is similar to those of the majority of the Curculionoidea [5, 6] in relation to structure and localization but the subacrosomal lamella was not observed between acrosome and nucleus, as described for other Curculionidae [5]. Crystalline arrangements of acrosomal contents have been found in various insects [4, 13, 15], but no such formation occurs in the acrosome of *C. sordidus*.

The nucleus, as in most Curculionoidea, is long and thin, containing densely compact and homogeneous chromatin. This is contrary to the description for *Stophilus oryzae* L., on which the taxonomic characteristics of the subfamily Rhynchophorinae has been established. This species is claimed not to have a compact nucleus [5].

Mitochondrial derivatives of different sizes have been reported for all known Curculionoidea [6-9, 16]. The internal structures of the two mitochondrial derivatives are similar to those described for *S. multistriatus* [16] and various families of Curculionoidea [5]. The insertion of the major mitochondrial derivative into a cavity of the nucleus is the condition accepted for most Curculionoidea, with the exception of *S. oryzae* [5]. Again, in relation to this species, the mitochondrial derivative was described as being lateral in relation to the nucleus.

The axoneme has the typical arrangement of microtubules described for many insects [1, 3-5, 8, 11, 14]. However, nine accessory microtubules and one of the central microtubules contain dense material in *C. sordidus*, while other insects have dense material in both central microtubules, as well as in the nine peripheral elements [3, 11-13, 15].

The accessory bodies of *C. sordidus* are very similar to these structures in other Curculionoidea previously described [5]. They are not, however, a uniform feature in all coleopterans, often varying in shape, as in the rounded bodies, subdivided into distinct medullar and cortical regions, found in *Divales bipustulatus* [10]. They may even be lacking in *Dermestes frischii* [7].

The expansions of these bodies are less dense and of unequal size in most Curculionidae while in other species they are equal U-shaped as in *S. multistriatus* [9] and *Curculio elephas* [5]. This expansions may also not exist, as in *D. bipustulatus* [10].

The analysis of *C. sordidus* has shown that this species is very similar to the other families of Curculionoidea previously studied [5]. However this species differs from *S. oryzae* which was analysed as a representative of the Rhynchophorinae. In *C. sordidus*, the acrosome is implanted in an anterior depression of the nucleus, while this depression is much shallower or even non-existent in other Curculionidae. Also, there is no subacrosomal lamella in this species, as has been described for other members of the subfamily. The nucleus of *C. sordidus* is densely compacted and has a concavity at its base in which the larger mitochondrial derivative is anchored. This arrangement of the flagellar organelles is also in disagreement with the description of *S. oryzae*, where the mitochondrial derivative was found laterally placed in relation to a nucleus with diffuse chromatin.

In view of these conflicting features, we believe that other species should be investigated so as to confirm the structures which may be adopted as taxonomic characteristics of the Rhynchophororinae.

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